Staphylococcus Aureus-Antimicrobial Resistance Analysis in Dairy Farms

ABSTRACT

In India, dairy farming is run by medium, small, and landless farmers and has a stable biotope at the animal-human-environment interface. Hence a cross-sectional study was conducted for the detection of antimicrobial resistance in targeting indicator bacteria *Staphylococcus aureus* at the animal-human-environment interface. A total of 280 samples were collected from dairy farms and their environment. The highest prevalence was noticed in human sources (50%), followed by animal sources (28.50%), dairy equipment (27.50%), and the lowest in farm environments (20.83%), and the overall prevalence noted was 27.85%. The prevalenceofspecies-specific*sau*genefor*S. aureus*was 80.76%.Virulence-associatedgenesviz.*sea* and*seb*weredetectedin7.93% and9.52%isolatesof *S. aureus*. The thermostable nuclease *nuc* gene was found in isolates from animal and human sources with an overall prevalence of 41.26%. Overallantimicrobialresistancepatternof*S.aureus*isolatedfromdairy farm and its environment was in the descending order *i.e.,* highest for cefoxitin,erythromycin,amikacin,andclindamycin;moderateresistancewasseeninvancomycina ndstreptomycin,linezolidandtetracyclineandthelowestresistancewas observed in chloramphenicol, gentamicin, and amoxicillin-clavulanic acid irrespective of the source. In the present study, the prevalence of *tet*M was 20% (2/10) in farm environments, 1.75% (1/57) dairy animals, and 33.33% (1/3) in dairy equipment with an overall prevalence of 5.19%.

Keywords: *Staphylococcus aureus,* Virulence, Antimicrobial Resistance, Dairy Farms, Animal-Human-Environment Interface

1. INTRODUCTION

The dairy industry in India provides self-sustainability to millions of rural households contributing about 4.11% to India's GDP and 25.6% to agricultural GDP, in which the dairy sector claims a major share by contributing 67% to total livestock output. Sustainable and profitable dairy farming demands proper management practices involving healthy animals well well-managed sheds, feeding, cleaning and sanitation, worker's hygiene, biosecurity, manure disposal, and so on. Among these, disease management is mainly focused on regular vaccination, deworming, health check-ups, and the use of antimicrobial preparations for treatment, control, and prevention of diseases of dairy animals. Unfortunately, in recent years upsurge in the use of antimicrobial preparations has been noticed mainly due to self-medication by the farmers, unwanted overuse by the paravets or sometimes by veterinarians, and lack of observance of proper withdrawal period. This irrational and unscrupulous use promotes the microbiota to develop resistance to survive against antimicrobial preparations.

Due to the rise of antibiotic-resistant pathogens in human health, animal health as well as food production, WHO has declared that AMR is one of the top 10 global public health threats facing humanity (WHO, 2022). Transmission potential of antimicrobial resistance is through indirect consumption of food, water, and produce contaminated with antimicrobial-resistant pathogens and environment via direct contact with animals and animal waste. This is a critical concern at the animal-human environment interface, where animal-origin food can be contaminated and spread further. (Ruegg et al. 2015).

Dairy farming includes regular contact with animals during milking and handling as well as exposure to manure, dust, and liquid splashes, all of which contribute transmission potential of bacteria to people, dairy animals, equipment, and the surrounding farm environment indicating multiple drivers are responsible for dissemination of AMR across the farm. To strengthen knowledge about drivers of antibiotic-resistant bacteria, the present study has targeted indicator bacteria viz. *Staphylococcus* spp. as this bacterium shares microbial biotopes in both humans and animals (Holmes et al. 2016). *Staphylococcus* spp. being commensal bacteria, colonizes on soft tissues, and skin, in the udder or milk. Among all 63 species, *Staphylococcus aureus* is one of the major etiological agents of clinical and subclinical mastitis and has negative public health implications through food-borne intoxication. Enterotoxin production has been linked to sepsis-related infections, food poisoning, pneumonia, and toxic shock syndrome (Lowy 2003). The present cross-sectional study of dairy farms and their environment analyzes the magnitude to which dairy animals may contribute to the AMR of indicator bacteria at the animal-human-environment interface in the population associated with dairy farming.

2. MATERIAL AND METHODS

Eight dairy farms from ten villages in the Satara district of Maharashtra were identified. The farms having at least 10 milking animals at the time of milking (morning or evening) were considered for sampling. A total of 35 samples were collected from each farm from different sources such as animals, human, farm environment, and dairy equipment were collected and mentioned in Table -1.

Table no 1. Details of samples collected

Sr.No.	Sample source	Samples	Total samples
1	Animals	1. Faeces	10
		2. Milk	5
		3. Udder swab	10
2	Human	4. Hand swab	2
3	Farm Environment	5. Drinking water	2
		6. Drainage water	1
		7. Feed	1
		8. Farm air	1
		9. Floor swabs	1
4	Dairy equipments	10. Can swabs	2
Total samples from each farm			35
Total samples – 35 X 10 farms			350

Isolation of *Staphylococcus aureus* was carried out by the procedure as per the Bacteriological Analytical Manual (FDA 2019). Presumptive isolates were further subjected to Gram staining and biochemical tests viz. catalase test, DNAase test (Cheesbrough 2004), Voges-Prausker test, methyl red test and oxidase test (Cruckshank et al. 1975).Antibiogram sensitivity test of isolates was conducted using Kirby Bauer disc diffusion method (Bauer 1966) for evaluating resistance

pattern. Antibiotics used in this study were Erythromycin, Amikacin, Ciprofloxacin, Gentamicin, Tetracycline, Vancomycin, Clindamycin, Chloramphenicol, Cefoxitin, Streptomycin, Amoxycillin-Clavulanic acid and Linezolid. Inhibition zones were recorded and interpreted according to the manufacturer's instructions and CLSI guidelines (CLSI 2020).

Biochemically confirmed isolates were subjected to molecular confirmation for the detection of 16s-rDNA gene and species-specific *sau*gene using multiplex PCR as per protocol described by Strommenger et al. 2003 with slight modification. DNA was extracted by boiling and snap chilling method. Multiplex PCR assay was performed according to Mehrotra et al. 2000 for the genes encoding for staphylococcal classical enterotoxins A, B, C, and D. Multiplex PCR was carried for genes *van*A and *van*B encoding for vancomycin according to Hizlisoy et al. 2018. A multiplex PCR assay was performed for detection of *tet*K and *tet*M genes responsible for tetracycline resistance according to Kumar et al. 2010. All primer sequences used in this study are mentioned in Table No. 2. Amplified PCR products were confirmed on 1.5% of agarose gel stained with ethidium bromide.

Table no. 2 Primer sequences used in this study.

Gene	Primer	Oligonucleotide Sequence (3'-5')	Amplicon Size
16S-rDNA	16S-F	CAG CTC GTG TCG TGA GAT GT	420
	16S-R	AAT CAT TTG TCC CAC CTT CG	
S .aureus specific	*sau*-F	AAT CTT TGT CGG TAC ACG ATA TTC TTC ACG	107
	sau-R	CGT AAT GAG ATT TCA GTA GAT AAT ACA ACA	
Sea	*sea*-F	GGT TAT CAA TGT GCG GGT GG	102
	sea-R	CGG CAC TTT TTT CTC TTC GG	
Seb	*seb*-F	GTA TGG TGG TGT AAC TGA GC	164
	seb-R	CGT AAT GAG ATT TCA GTA GAT AAT ACA ACA	
Sec	*sec*-F	AGA TGA AGT AGT TGA TGT GTA TGG	451
	sec-R	CAC ACT TTT AGA ATC AAC CG	
Sed	*sed*-F	CCA ATA ATA GGA GAA AAT AAA AG	278
	sed-R	ATT GGT ATT TTT TTT CGT TC	
Nuc	*nuc*-F	AGT TCA GCA AAT GCA TCA CA	400
	nuc-R	TAG CCA AGC CTT GAC GAA CT	
*van*A	*van*A-F	GTA GGC TGC GAT ATT CAA AGC	231
	*van*A-R	CGA TTC AAT TGC GTA GTC CAA	
*van*B	*van*B-F	GTA GGC TGC GAT ATT CAA AGC	330
	*van*B-R	GCC GAC AAT CAA ATC ATC CTC	
*tet*K	*tet*K-F	GTA GCG ACA ATA GGT AAT AGT	360
	*tet*K-R	GTA GTG ACA ATA AAC CTC CTA	
tetM	*tetM*	AGTGGAGCGATTACAGAA	158
	tetM	CATATGTCCTGGCGTGTCTA	

3. RESULTS AND DISCUSSION

Isolation of *S. aureus* isolates was conducted according to the Bacterial Analytical Manual (FDA 2019) and streaking on selective media Baird-Parker media. The prevalence *of S. aureus* in 8 different dairy farms was in the order with an overall prevalence of 27.85%. Among the 280 samples collected from 4 different sources, the highest prevalence was found among the dairy workers (50.00%), followed by the dairy animals (28.50%), followed by farm environment (20.83%), and lowest in dairy equipment (18.70%). Within the animal source, the highest prevalence was seen in milk (52.50%), followed by udder (31.25%), and the lowest seen in fecal samples (13.70%). Within the farm environment, the prevalence of S. aureus was in the decreasing order of drainage water (37.50%), floor swabs (25%), and equal prevalence in drinking water, feed, and farm air (12.50%).

The presence of bacteria in dairy farms indicates potential cross-transmission from dairy animals and dairy farm workers and its environment recirculation through contaminated feed, air currents, drinking water, and drainage. In the study, milk and dairy farm workers followed by udder swabs were found to be potential reservoirs of bacterium. contamination of the environment indicated its transmission and survival potential. Earlier studies related to the prevalence of *S. aureus* have mentioned wider variation. Gwida et al. (2021) found a high prevalence of 59.30% in dairy animals, 100% in milk, 50.00% in teat swabs, and 43.50% in fecal samples. Prevalence in fecal samples was slightly lower than the prevalence noted by Badaway et al. (2022). Multiples of authors like Lee et al. (2012), Liu et al.(2018), Regasa et al. (2019), Thakur et al. (2020), Tibehu et al. (2021), Banu

&Geberemedhin(2022) and Liu et al. (2022) noted prevalence in milk in the range 15.00% to 58.33%. The highest prevalence (82.50%) in dairy farm workers was reported by Gwida et al. (2021), while lowest (3.33%) was recorded by Lee et al. (2012). Whereas other studies showed prevalence of 25.00%, 22.90%, 19.23%, 16.51% and 7.00% by Regasa et al. (2019), Liu et al. (2018), Tibebu et al. (2021), Banu&Geberemedhin (2022). A prevalence of 35.42% reported by Liu et al. (2022) in the sewage samples from dairy goat farms, 27.78% in the soil of the floor and 7.50% in feed nearly matches with present cross-sectional study. Deddefo et al. (2023) noted a 10% prevalence in water used for cleaning udder and milkers' hands which matches with the present study, while Ganai et al. (2015) reported a 46.66% prevalence of the bacterium in floor swabs which was a higher as compared to this study. Many authors reported variable presence of *S. aureus* in dairy equipment as Banu&Geberemedhin (2022) found a 12.73% prevalence and Deddefo et al. (2023). Tibebu et al. (2021) noted the prevalence of *S. aureus* at different farms in the range of 15.79% to 58.33%.

1.1 Molecular confirmation of *S. aureus*

For molecular confirmation of *S. aureus,* biochemically confirmed isolates were subjected to detect genus-specific 16S-rRNA and species-specific *sau* gene using multiplex PCR phenotypically positive isolates were confirmed genotypically using 16S-rDNA, however, 63 isolates carried *sau* gene showing overall 80.76% molecular prevalence of *S. aureus*. 47 out of 57 (82.45%) isolates including 7 out 11 fecal (63.63%), 15 out of 21 milk (71.42) and 25 out 25 (100%) isolates from udder swabs from the animal source were confirmed for the presence of *sau* gene. 8 isolates (100%) from dairy farm workers were positive for *S. aureus* at molecular level. From farm environment, 6/10 isolates harboured *sau* gene including one each from drinking water, drainage water, feed and farm air, and two from floor swabs. Dairy equipment swab isolates showed 2 out 3 bacteria possessing *sau* gene. Presence of *sau* gene is considered gold standard for identification of *S. aureus* (Hanon 2017). In the present study, genotypic confirmation of the isolates was carried out using 16S-rRNA and *sau* gene, which confers coagulase gene detection.

1.2 Characterisation of virulent determinant genes of the isolates.

In the present study, most potent enterotoxin expressing genes viz. *sea, seb, sec* and*sed* were screened by the protocol given by Mehrotra et al. (2000). Overall prevalence of virulence gene *sea* and *seb*was 7.93% (5/63) and 9.52% (6/63), respectively. The prevalence of virulent gene sea was 7.01% (4/57) and 12.8% (1/8) from dairy animal and dairy farm workers, respectively and within the animal source, *sea* producing isolates were from milk (2) and udder-swabs (2), while the prevalence of virulent gene *seb* was 7.01% (4/57) and 25.00% (2/8) from dairy animal and dairy farm workers, respectively, within the animal source, *sea* producing isolates were from milk (1) and udder swabs (3). Even et al. 2009 stated expression of SEs gene is linked to the Agr-related quorum sensing system to host cell interaction. In the present study, SEs genes were expressed by the isolates from milk, udder swabs, and dairy farm workers. As enterotoxin production is type dependent and host-pathogen interaction and *L. lactis* competes with *S. aureus* for expression of *sec* and *sed*(Even et al. 2009)*, it could be correlated with* expression of only *sea* and *seb* is seen in the current study. Present enterotoxin prevalence of *sea* was slightly lower than Liu et al. (2018). Similar pattern was observed in the case of *seb* also. The prevalence of *seb* observed in hand swabs was nearly equal to the prevalence noted by Zeinhom et al. (2015). In the present study, *sea* and *seb* were found in 5 isolates from udder swabs.

1.3 Antibiogram study of *S. aureus* isolates

All biochemically confirmed isolates were subjected to an antibiogram study using Kirby Bauer disc diffusion method (Bauer, 1966) and interpreted based on CLSI guidelines (CLSI 2020). Resistance to Cefoxitin was found in samples from every source except in dairy equipment, indicating dairy farm workers and dairy animals acting as main drivers' further spreads to the environment and equipment. Its cross-resistance is due to penicillin and cephalosporin mainly through the *mec*A gene. The resistance pattern for erythromycin, being the highest resistance in dairy animals and dairy equipment followed by equal resistance in dairy farm workers and the environment, highlighted transmission from dairy animals and dairy equipment to dairy farm workers and the environment. Antibiotics of class macrolide used at the field level were found to be azithromycin not erythromycin and Gagliotti et al. (2006) highlighted cross-resistance to erythromycin due to azithromycin. The aminoglycosides group *i.e.,* amikacin and streptomycin showed the highest resistance in dairy farm workers, but another antibiotic gentamicin was found to be the least resistance in each of the sources. In the present study, it was noticed that transmission of resistance for aminoglycosides had equally contributed by dairy animals and dairy farm workers and its persistence in its environment reflected hygienic condition of dairy animals and dairy farm workers. Antibiogram profile for clindamycin, highest resistance was seen in isolates from dairy animals indicating its source spread to dairy equipment, dairy farm workers and dairy environment. Resistance to vancomycin is of utmost

importance. Moderate resistance was found in dairy animals' environment and dairy equipment. But among these, major resistance was observed in dairy animals indicating its source even though its use in veterinary practice is still not found. More interestingly, its use in human medicine was noticed but zero resistance was seen. This contrasting result gained immense importance practical point of view. With future aspect, its surveillance should be conducted time to time. 40% isolates from dairy environment mainly drainage water, farm air and floor swabs were found to be tetracycline resistant which may predict that dairy animals from previous production may be harboured and shed tetracycline resistant bacteria in dairy environment. Thus, dairy environment was acting as main driver. Antibiogram studies conducted by Dweba et al. (2019) revealed highest resistance to penicillin G followed by cefoxitin and erythromycin and lowest resistance to ciprofloxacin and gentamicin which shows similarities with current studies.Liu et al. (2018) also found moreover similar resistance patterns irrespective of the source. Ganai et al. (2015) resistance to penicillin G (68.75%), ampicillin (65.625%), and streptomycin (59.375%) in the dairy environment. Akindolire et al. (2015) and Liu et al. (2018) noted similar resistance patterns for erythromycin but Mbindyo et al. (2021) noted quite lower than the present study. Overall, the studies consistently show significant levels of resistance to penicillin, ampicillin, tetracycline, erythromycin, and other commonly used antibiotics. Antibiotic resistance noted by Juwita et al. (2022) from human source isolates in dairy farms was found to be in contrast to the current study, due to variation in the selection of antimicrobial preparations. The antibiogram study conducted by Ganai et al. (2015) is quite similar concerning amikacin, but higher than the current study for streptomycin and gentamicin.

3.4 Detection of antimicrobial resistant genes

In the present study, tetracycline-resistant genes (*tet*K and *tet*M), vancomycin-resistant gene (*van*A and *van*B) were screened. In this study, neither *van*A nor *van*B were expressed in the isolates. In the present study, the prevalence of *tet*M was 20% (2/10) in farm environments, 1.75% (1/57) in dairy animals, and 33.33% (1/3) in dairy equipment with an overall prevalence of 5.19%. The *tet*K gene was not harbored by any one of the isolates. In the present study, the *van*A and *van*B genes responsible for vancomycin resistance were expressed in any of the isolates and these results match with Bhattacharya et al. (2016) who also noticed the absence of these genes. However, Qu et al. (2019) found the *van*A gene in 4% of staphylococcal isolates from bovine clinical mastitis but were unable to detect the *van*B gene. In contrast, Bissong and Ateba (2020) detected the *van*B gene in 5 isolates but *van*A was not detected. So also, Hizlisoy et al. (2018) showed an 11.00% prevalence of the *van*B gene but did not find the *van*A gene in *S. aureus* isolates. Similarly, Hizlisoy et al. (2018) and Liu et al. (2018) found a more predominant *tet*M gene than *tet*K which agrees with the findings of the present study. Antibiogram studies have shown intermediate to high resistance and this difference in phenotypic and genotypic resistance profile might be attributed to the presence of other plasmid-mediated tetracycline resistant determinants viz. *tet*L, *tet*N, *tet*Q, *tet*W, *tet*A, *tet*B and *tet*C possibly transferred to collected isolates (Jahantigh et al. 2020; Leroy et al. 2019).

4. CONCLUSION

Presence of indicator organisms viz. S. aureus in dairy farms along with thedevelopment of antimicrobial resistance, exhibited phenotypically as well as genotypically, raises significant public health concern with a possibility of cross-contamination from animal to human and vice-versa and from environment too. The presence of classical enterotoxins from animal and human source samples indicates public threat directly as milk is consumed by each age group of the community. The possibility of transmission potential of antimicrobial-resistant genes cannot be rejected although they were not exposed directly. Genotypic profiling of antimicrobial-resistant genes should be investigated thoroughly including multiple resistant determinants.

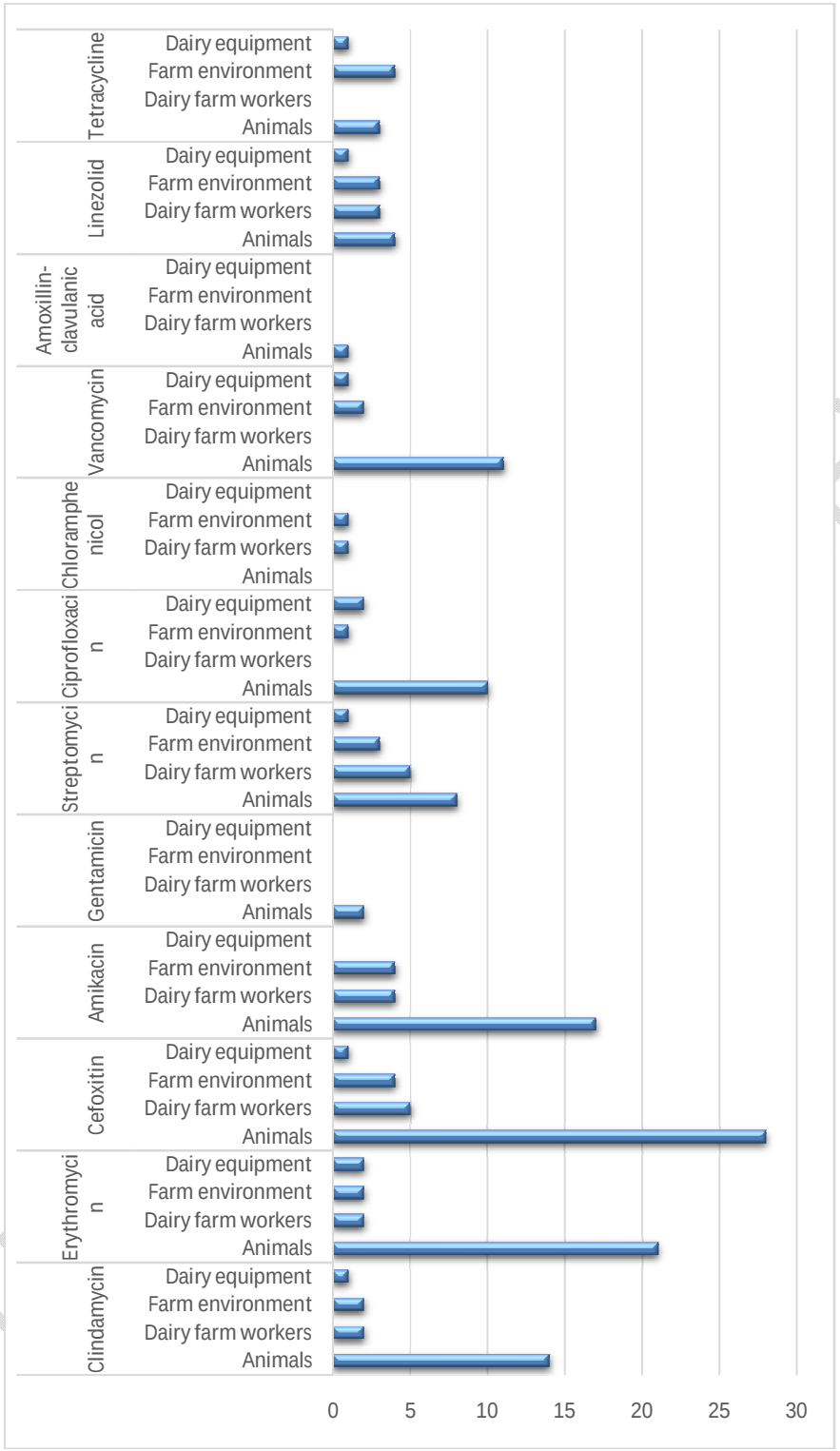

Fig. 1.Overall antimicrobial resistance pattern of *S. aureus*

REFRENCE

Akindolire, M. A., Babalola, O. O. & Ateba, C. N. (2015). Detection of antibiotic resistant *Staphylococcus aureus* from milk: A public health implication. International Journal of Environmental Research and Public Health, 12 (9), 10254–10275.

Badawy, B., Elafify, M., Farag, A. M. M., Moustafa, S. M., Sayed-Ahmed, M. Z., Moawad, A. A., Algammal, A. M., Ramadan, H. &Eltholth, H. (2022). Ecological distribution of virulent multidrug-resistant *Staphylococcus aureus* in livestock, environment, and dairy products. Antibiotics, 11(11), 1651.

Banu, M. G., & Geberemedhin, E. (2022). Occurrence and antimicrobial susceptibility of *Staphylococcus aureus* in dairy farms and personnel in selected towns of West Shewa Zone, Oromia, Ethiopia. PLoS One. 17 (11), e0277805.

Bauer, A. W. (1966). Antibiotic sensitivity testing by a standardized disc diffusionmethod.American Journal of Clinical Pathology. 45 (4), 149-158.

Bhattacharyya, D., Banerjee, J., Bandyopadhyay, S., Mondal, B., et al. (2016). First report on vancomycin-resistant Staphylococcus aureus in bovine and caprine milk. Microbial drug resistance, 22 (8), 675-681.

Cheesbrough, M. (2004). District laboratory practice in tropical countries. Part, *2*, 348-3612000.

CLSI. (2020). Performance standards for antimicrobial susceptibility testing: twentyeighth information supplement. M 100- S28. 30th Ed. Clinical LaboratoryStandardsInstitute.

Cruikshank, R., Duguid, J. P., Marmion, B. P., & Swain, H. A. (1975). Medical Microbiology: The Practice of Medical Microbiology.

Deddefo, A., Mamo, G., Asfaw, M., & Amenu, K. (2023). Factors affecting the microbiological quality and contamination of farm bulk milk by Staphylococcus aureus in dairy farms in Asella, Ethiopia. BMC Microbiology, 23 (1), 65.

Even, S., Charlier, C., Nouaille, S., Ben Zakour, N. L., Cretenet, M., et al. (2009). *Staphylococcus aureus* virulence expression is impaired by Lactococcus lactis in mixed cultures. Applied and Environmental Microbiology, 75 (13), 4459-4472.

FDA. (2019).BacteriologicalAnalyticalManual,https://www.fda.gov/food/laboratory-methods-food/bam-chapter-12-Staphylococcus aureus.

Ganai, A. W., Kotwal, S. K., Malik, M. A., Sharma, H. K., Wani, N., & Jeelani, R. (2015). Prevalence of Staphylococcus aureus and Methicillin Resistant Staphylococcus aureus (MRSA) in clinical setting and dairy farm environment of Jammu. Journal of Animal Research, 5 (3), 539-545.

Gwida, M. M., Saad, T. M., Elgohary, A., & Mohamed, A. (2021). Characterization of methicillin-susceptible and methicillin-resistant Staphylococcus aureus from healthy cattle and buffaloes in a linked community. Mansoura Veterinary Medical Journal, 22(2), 76-81.

Hanon, B.M. (2017). Molecular study of some virulence genes in biotype diversity of methicillin resistance Staphylococcus aureus isolated from handling carrier and bovine mastitis. International Journal of Science and Research. 6(2): 1746-1752.

Hızlisoy, H., Ertaş Onmaz, N., Karadal, F., Al, S., Yıldırım, Y., Gönülalan, Z., & Kılıç, H. N. (2018). Antibiotic resistance gene profiles of Staphylococcus aureus isolated from foods of animal origin. Kafkas Universitesi Veteriner Fakultesi Dergisi, *24*.

Holmes, A. H., Moore, L. S., Sundsfjord, A., Steinbakk, M., et al. (2016). Understanding the mechanisms and drivers of antimicrobial resistance. The Lancet, *387*(10014), 176-187.

Jahantigh, M., Samadi, K., Dizaji, R. E., & Salari, S. (2020). Antimicrobial resistance and prevalence of tetracycline resistance genes in Escherichia coli isolated from lesions of colibacillosis in broiler chickens in Sistan, Iran. BMC veterinary research, *16*, 1-6.

Juwita, S., Indrawati, A., Damajanti, R., &Mayasari, N. (2022). Multiple antibiotic resistance and virulence factors of Staphylococcus aureus strains isolated from dairy farms in South Sulawesi, Indonesia. *Biodiversitas:* Journal of Biological Diversity, 23(2) 1015-1022.

Kumar, R., Yadav, B. R., & Singh, R. S. (2010). Genetic determinants of antibiotic resistance in Staphylococcus aureus isolates from milk of mastitic crossbred cattle. Current Microbiology, *60*, 379-386.

Lee, S. H. I., Camargo, C. H., Gonçalves, J. L., Cruz, A. G., Sartori, B. T., Machado, M. B., & Oliveira, C. A. F. D. (2012). Characterization of *Staphylococcus aureus* isolates in milk and the milking environment from small-scale dairy farms of São Paulo, Brazil, using pulsed-field gel electrophoresis. Journal of Dairy Science, *95*(12), 7377-7383.

Leroy, S., Christieans, S., & Talon, R. (2019) Tetracycline gene transfer in *Staphylococcus xylosus in situ* during sausage fermentation. Frontiers in Microbiology, 10, 392. doi.org/10.3389/fmicb.2019.00392

Liu, B., Sun, H., Pan, Y., Zhai, Y., et al. (2018). Prevalence, resistance pattern, and molecular characterization of *Staphylococcus aureus* isolates from healthy animals and sick populations in Henan Province, China. Gut pathogens, *10,* 1-13.

Liu, H., Dong, L., Zhao, Y., Meng L, et. al. (2022). Antimicrobial susceptibility and molecular characterization of *Staphylococcus aureus* isolated from different raw milk samples in China. Frontiers in Microbiology, 13, 840670. doi.org/10.3389/fmicb.2022.840670

Lowy, F.D., (2003). Antimicrobial resistance: The example of *Staphylococcus aureus*. The Journal of Clinical Investigation, 111(9), 1265-1273.doi:10.1172/JCI200318535.

Mbindyo, C.M., Gitao, G.C., Plummer, P.J., Kulohoma, B.W., et al. (2021). Antimicrobial resistance profiles and genes of staphylococci isolated from mastitic cow's milk in Kenya. Antibiotics, *10*(7), 772. doi.org/10.3390/antibiotics10070772

Mehrotra, M., Wang, G., & Johnson, W.M. (2000). Multiplex PCR for detection ofgenesfor*Staphylococcusaureus*enterotoxins,exfoliativetoxins,toxicshocksyndrometoxin1,and methicillinresistance.JournalofClinicalMicrobiology,38(3), 1032-1035.

Phiri, B.S.J., Hang'ombe, B.M., Mulenga, E., Mubanga, M., et al. (2022). Prevalence and diversity of *Staphylococcus aureus* in the Zambian dairy value chain: A public health concern. International Journal of Food Microbiology, 375: 109737. doi.org/10.1016/j.ijfoodmicro.2022.109737

Qu, Y., Zhao, H., Nobrega, D.B., Cobo, E.R., et al. (2019). Molecular epidemiology and distribution of antimicrobial resistance genes of *Staphylococcus* species isolated from Chinese dairy cows with clinical mastitis. Journal of Dairy Science, *102*(2), 1571-1583.

Regasa, S, Mengistu, S., & Abraha, A. (2019). Milk safety assessment, isolation, and antimicrobial susceptibility profile of *Staphylococcus aureus* in selected dairy farms of Mukaturi and Sululta Town, Oromia Region. *Ethiopia.* Veterinary Medicine International,3063185. doi.org/10.1155/2019/3063185

Ruegg, P.L., Oliveira, L., Jin, W., &Okwumabua, O. (2015). Phenotypic antimicrobial susceptibility and occurrence of selected resistance genes in Gram-positive mastitis pathogens isolated from Wisconsin dairy cows. Journal of Dairy Science *98*, 4521–4534. doi.org/10.3168/jds.2014-9137

Sonali Thakur, M.N. Brahmbhatt, J.H. Chaudhary, B.C. Parmar, U.P. Mistry and C.D. Bhong (2020). Comparison of Loop-mediated isothermal amplification with polymerase chain reaction for detection of methicillin-resistant *Staphylococcus aureus* in Chevon. Journal of Entomology and Zoology Studies. Vol. 8 (6): 1976-1980. https://doi.org/10.22271/j.ento.2020.v8.i6aa.8111

Tibebu, L., Belete, Y., Tigabu, E. & Tsegaye, W. (2021). Prevalence of *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* and potential risk factors in selected dairy farms at the interface of animal and human in Bishoftu, Ethiopia. Veterinary Medicine: Research and Reports, 241-251.

WHO (2022) WHO Strategic and Technical Advisory Group for Antimicrobial Resistance (STAGAMR): Report of the second meeting, 14-16 June 2022. Geneva: World Health Organization; 2022. Licence: CC BY-NC-SA 3.0 IGO.

Zeinhom, M.M., Abdel-Latef, G.K. & Jordan, K. (2015). The use of multiplex PCR to determine the prevalence of enterotoxigenic *Staphylococcus aureus* isolated from raw milk, feta cheese and hand swabs. Journal of Food Science,*80*(12), M2932–M2936. https://doi.org/10.1111/1750-3841.13147.